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GENOME ANNOUNCEMENT

Complete Genome Sequences of Newcastle Disease Virus Strains Circulating in Chicken Populations of Indonesia

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Eight highly virulent Newcastle disease virus (NDV) strains were isolated from vaccinated commercial chickens in Indonesia during outbreaks in 2009 and 2010. The complete genome sequences of two NDV strains and the sequences of the surface protein genes (F and HN) of six other strains were determined. Phylogenetic analysis classified them into two new subgroups of genotype VII in the class II cluster that were genetically distinct from vaccine strains. This is the first report of complete genome sequences of NDV strains isolated from chickens in Indonesia.

Newcastle disease (ND) is a highly contagious viral disease of poultry. ND was first reported in Java, Indonesia, in 1926. Although all commercial chickens in Indonesia are routinely vaccinated with live Newcastle disease virus (NDV) vaccines, ND continues to be a major problem for the poultry industry (7). NDV is a member of the genus *Avulavirus* in the family *Paramyxoviridae*. The genome of NDV is single-stranded, negative-sense, nonsegmented RNA with six genes encoding seven major viral proteins (3). NDV strains are divided into two classes based on genetic analysis: class I strains have been isolated mainly from wild birds and are generally avirulent, whereas class II strains have been recovered from wild and domestic birds and include virulent and avirulent isolates (2). Class I and II viruses are divided into 9 and 11 genotypes, respectively (4). To date, no complete genome sequences have been reported for NDV strains isolated from chickens in Indonesia. Only two partial F gene sequences (315 and 395 nucleotides) were available (1).

In 2009 and 2010, outbreaks of ND occurred in commercial chickens in Indonesia, causing up to 70% to 80% mortality. The sequences of the F and HN genes of eight NDV isolates were determined by reverse transcription (RT)-PCR using overlapped consensus primers and direct sequencing. The 3′ and 5′ termini were determined by rapid amplification of cDNA ends (RACE) (8). The amino acid sequence identities of the F and HN proteins among these eight strains ranged from 96% to 100% and 93% to 100%, respectively, compared to 87% and 89% between the Indonesian strains and the vaccine strains B1 and LaSota. This indicates that the circulating strains were substantially distinct from the vaccine strains in use and suggests that antigenic differences contributed to poor vaccine protection.

The sequence of the F protein cleavage site is a major determinant of NDV pathogenicity. The cleavage sites of virulent NDV strains usually contain multiple basic residues, whereas avirulent strains have fewer basic residues (5, 6). The eight Indonesian strains had two cleavage-site sequences that were each consistent with a virulent pathotype: RRQKR ↓ F and RRRKR ↓ F (underlining represents the basic residue; arrow represents the site of cleavage). Phylogenetic analysis of the F and HN genes of the eight strains constructed by MEGA4.0 indicated that they represent two new subgroups within genotype VII in class II.

Complete genome sequences were determined for two of these Indonesian NDV strains, namely, Banjarmasin/010/10 and Sukorejo/019/10, each representing one of the two new subgroups. The genomes of both Indonesian strains are 15,192 nucleotides in length. Comparison of genome sequences demonstrated 92% nucleotide sequence identity between the two Indonesian strains compared with 82% identity between either Indonesian strain and the vaccine strain B1 or LaSota (note that both vaccine strains are genotype II of class II and share 99% identity). The data described here provide evidence of divergence in circulating virulent strains within an outbreak as well as between these circulating and vaccine strains.

Nucleotide sequence accession numbers. The following genome sequences have been deposited in GenBank: Banjarmasin/010/10 (HQ697254), Sukorejo/019/10 (HQ697255), Makassar/003/09 (HQ697256), Gianyar/013/10 (HQ697257), Sragen/014/10 (HQ697258), Kudus/017/10 (HQ697259), Kudus/018/10 (HQ697260), and Bali/020/10 (HQ697261).

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